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ORIGINAL PAPER

On Valorization of Brewer's Yeast as an Environmentally Sustainable Fishmeal Replacement in *Labeo rohita* Nutrition: Insight to Growth Attributes, Digestive Enzyme Activities and Haemato-biochemical Indices

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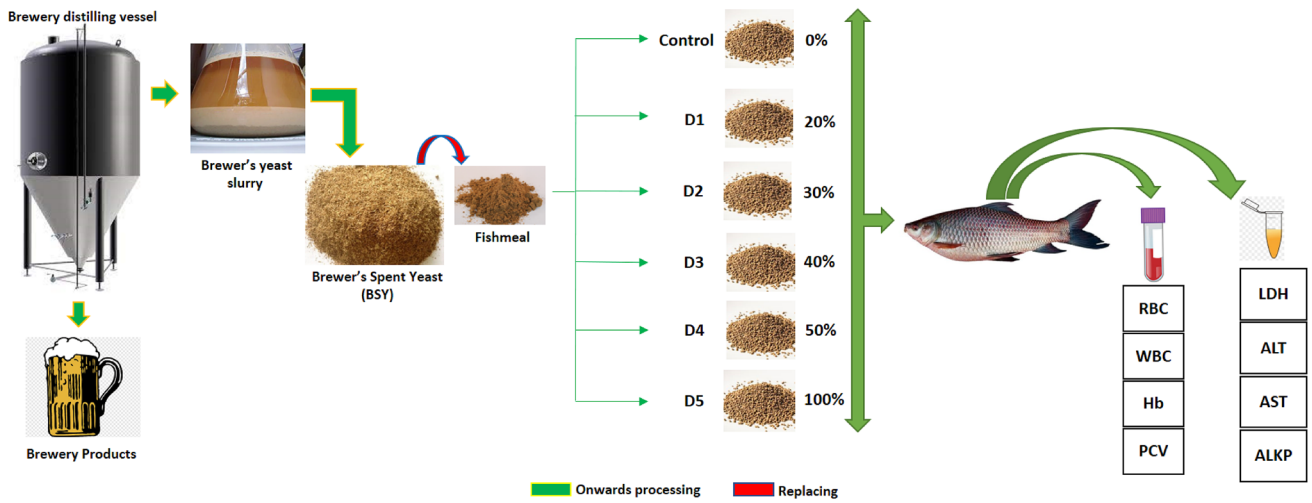
Abstract

Fishmeal replacement in aquafeed is a prime challenge for researchers in the modern era of fish nutrition. Six isonitrogenous diets (27% protein) were prepared with graded fishmeal replacement i.e., 0% (Control), 20% (D1), 30% (D2), 40% (D3), 50% (D4) and 100% (D5) by brewer's spent yeast (BSY). The efficiency of these diets was evaluated on *Labeo rohita* (2.0 ± 0.02 g) fingerlings in 90-day feeding trial under controlled environmental conditions of 250 L FRP tanks. All treatments were having three replicates and fed with 4% body weight. The result revealed that 30% replacement of fishmeal showed significantly higher values in terms of growth attributes like weight gain, average daily growth, specific growth rate and survival (%) than that of control and other BSY incorporated diet. The feed utilization in terms of feed conversion ratio, feed conversion efficiency, protein efficiency ratio and apparent net protein utilization was significantly better in D2, followed by D3. The highest value of RBC, haemoglobin and packed-cell volume was found in D2, whereas that of WBC was found in D5. The amylase activity was highest in D1 whereas lipase and protease were highest in D2. The level of cortisol, glucose, alkaline phosphatase and lactate dehydrogenase was lowest in D2. Both liver enzymes such as alanine aminotransferase and aspartate aminotransferase were also significantly lower in D2 and gradually increased with the inclusion of brewer's yeast. A similar trend was observed in serum protein, albumin and globulin level. Hence the study infers that a 30% replacement of fishmeal with brewer's spent yeast can significantly induce growth performances and immunity in *L. rohita*.

Graphical Abstract



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Keywords *Labeo rohita* · Brewer's spent yeast · Hematology · Digestive enzymes · growth attributes

Statement of Novelty

The intensive use of aquafeed has increased the demand of fishmeal protein, which can be met cheaply by BSY (brewer's spent yeast) and proved to be nutritionally efficient for Labeo rohita by 30% replacement of fishmeal. Brewer's spent yeast can be used as a source of feed ingredients for the aquafeed industry. The proper handling of these wastes may have positive economic effects and preventing environmental damage

Introduction

Fishmeal comprising an excellent nutrient profile is considered a gold standard for dietary protein sources within the livestock production sector and it has also covered widespread expansion as well as acceptance among the aquafeed industries [1]. Due to favourable nutritional qualities concerning aquaculture species acquired by the inclusion of fishmeal, aquafeed industries rely mostly on it for complete feed preparation [2]. However, limited availability and extremely high demand have made the commodity very expensive. Fishmeal is now considered environmentally unsustainable; hence, the rising demand for aquaculture forced the quest for alternative protein sources [3]. With the growing rate of fishmeal uses, Hardy [4] inferred that the industry might run out of a sufficient quantity of fishmeal. The suitable replacement for fish meal is presently a major research activity throughout the world to reduce the aquafeed cost. The development of a cost-effective diet for a candidate aquaculture species is of great concern. Many authors have experimented with partial or complete replacement of fish meal in aquafeed using several alternative feed ingredients either of plant or animal origin [5–8]. In earlier studies, specific strategies and techniques have been described to optimize the nutritional composition of plant-based feed and limit the potential adverse effects of bioactive compounds [9].

Brewery waste is a major by-product generated from distillery industries that manufacture and process beer [10, 11]. Among all the alcoholic beverages, beer is considered the most popular and the third most consumed beverage besides water and tea in the world [12]. However, such an immense amount of brewery waste production requires safe disposal, which is a prime concern for modern brewery industries. These wastes are environmentally hazardous and complete the oxidizing process demands 30–60% oxygen [13]. Some of the residues and byproducts such as spent grain hops, and yeast are prime examples of such distillery wastes [14]. The most common types of residues generated from the brewery are usually a good source of protein as well as essential amino acids [15]. The grains are processed through the mashing step where the drying and the crushing process occur and subsequently, the mixture is treated with 70–74 °C hot water, where the grain starch is converted to fermentable sugars [16]. The filter sugar-rich liquid also known as the wart is fermented to produce beer and the rejected material includes spent brewer's yeast. Since the intensification of a green environment has manifested global outreach, several researchers have focused on getting breakthroughs to turn brewery waste into certain beneficial use [16–19]. The Brewer's spent yeast (BSY) is a potential feed ingredient for use in animal and aquafeed due to its richness in protein along with immune stimulants like supplementing probiotics, nucleic acid, manganese and beta-glucan [10–14, 10, 20]. BSY is available throughout the year at low or no cost as a potential raw material for any potential application. In various fish species, a partial yeast-based diet has been reported to perform better than a fish meal-based diet [14–17, 21].

Rohu (*Labeo rohita*), Catla (*Catla catla*), and Mrigal (*Cirrihinus mrigala*) are the three major freshwater fish species in Indian sub-continents [22], among which rohu features outstanding market value and demand [23]. The intensification of fish culture has led to dependence on formulated feed. Protein is the most expensive and indispensable component in fish feed which primarily influence moderation in the growth of fish and the feed cost. At the same time, reducing feeding costs could be a key factor for the successful development of intensive aquaculture practices.

Haematological profile and biochemical indices have been considered diagnostic indices of pathological conditions and are important for the assessment of systemic functions and the overall health of animals. These parameters are related to the feed intake and energy requirement of fish. Fishes are usually in close relationship with the aquatic environment thus the blood will reveal conditions within the body of the fish long before there is any visible manifestation of the disease [24, 25–27]. Digestive enzyme activities vary within species and influenced by biotic (size, age, origin) and abiotic (temperature, season, food) factors which can alter enzyme activity levels. Diverse comparative studies of digestive enzymes in different fish species have been reported [25].

Information about the efficiency of BSY to replace fishmeal in the diet of Indian major carp, particularly rohu, is

scarce. In the present study, fishmeal has been replaced with BSY in the aquafeed at a graded level in order to evalute its suitability in terms of growth attributes, hemato-biochemical parameters and enzyme activities of *L. rohita*.

Theory

The brewing industry generates a substantial amount of waste of which brewer's spent yeast can be realized as a potential source of feed ingredients for the aquafeed industry. Proper management of these wastes may bring economic benefits and help to protect the environment from pollution caused by their excessive accumulation. At the same time, the global supply of forage fish reached its plateau, fed aquaculture must continue with an aim to reduce dependence on fishmeal to ensure sustainable sectoral growth. Keeping the above facts, the present study demonstrated that partial replacement of fish meal by brewer's spent yeast (30%) enhanced the growth without compromising the fish health. Hence, the by-products upcycled in aquaculture feed could substantially reduce the forage fish demand.

Materials and Methods

Experimental Fish and Design

The experiment was carried out for a period of 90 days. Rohu fingerlings were obtained from ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India. 30 rohu fingerlings (2.0 ± 0.02 g) were randomly distributed in indoor FRP tanks (3.2 m × 2 m × 2 m) having a water holding capacity of 250 L in triplicate. The experimental fish were acclimatized for 15 days and fed with control diet before commencing the experiment. The temperature was maintained at 27 °C using thermostatically controlled electric heaters and continuous aeration was provided to each tank to maintain the dissolved oxygen [26]. The faecal matter was cleaned and 30% of water was exchanged daily. The pH and dissolved oxygen content of the water were measured regularly and found to be between 7.3 and 7.6 and 5.9–7.1 mg L^{-1}, respectively. Fish were fed @ 4% of their body weight in split doses twice daily at 9.00 and 15.00 h.

Brewer's Spent Yeast (BSY)

Brewer's yeast slurry was obtained from United breweries, Industrial estate, Odisha, India and transported to the laboratory in plastic containers of 10 L capacity and oven-dried at 50 °C to get a constant weight. The oven-dried semi-solid liquid slurry centrifuged at 5000 rpm for 5 min, the surface slurry was collected and the supernatant was discarded. The precipitated solid residue was washed using sterilized distilled water and quickly filtered through 100 micron filter paper using a suction filter. Further the solid residues were spreaded on aluminium trays and transferred to an oven at 50 °C for 16 h. The subsequently obtained product was termed BSY, and used in feed preparation. Heat treatment of yeast at 50 °C in the oven promotes autolysis of the yeast cells. This optimized temperature is employed to release α-amino nitrogen during the process of yeast autolysis (Tanguler and Erten, 2008). The proximate composition of the dried yeast was determined using standard methods [27, 28] and compared with values in the literature and used in the feed formulation. The obtained proximate composition of BSY was protein: 52.71%, lipid: 3.16% and ash: 6.17.

Experimental diet

Six iso-nitrogenous (26.29–27.09%) and iso-caloric (1297.04 kJ/100 g) diets were prepared by replacing fishmeal with BSY at a graded level. Rice bran (protein 16.33% and lipid 5.27%), Groundnut oil cake (GNOC) (protein 51.17% and lipid 12.36%), fishmeal (protein 62.79% and lipid 6.76%), wheat (protein 12.58% and lipid 3.15%) and BSY (protein 52.71%and lipid 3.16%) were used as the major ingredients for the preparation of experimental diets. The energy level was adjusted by the contribution of fish oil (Seven Seas, Ireland, UK) and vegetable oil (Agro Tech Food Ltd, India). Vitamin and mineral mixture was added at 1% of the feed and the carboxy methyl cellulose (CMC) was used as the binder. The diet without addition of BSY was considered as the control diet. The fishmeal was replaced by BSY at a level of 20, 30, 40, 50 and 100% and termed as D1, D2, D3, D4 and D5 respectively. The proximate composition of the diet was analyzed following standard methods [27, 28] and is given in Table 1. The energy was calculated based on standard fuel values of 16.736, 16.736 and 37.656 kJ per g for protein, carbohydrate and lipid respectively [29]. Before feed preparation, for each diet, the ingredients were taken in required quantities along with oil and blended in an electrical grinder to prepare the dough. The dough was palletized following standard method [27] and stored in airtight, nitrogen-filled containers at normal room temperature until use.

Growth Attributes and Feed Utilization

The growth particulars such as average daily gain (ADG %), feed conversion ratio (FCR), feed conversion efficiency (FCE), the protein efficiency ratio (PER), specific growth rate (SGR %/day) and apparent net protein utilization (ANPU %) were calculated using standard formulae [27]. The initial and final carcass compositions of the 10

Table 1 Formulation and proximate composition of feed ingredients and experimental diets

Ingredients	Experimental diets					
	Control	D1	D2	D3	D4	D5
Rice bran (g)	33.142	33.142	33.142	33.142	33.142	33.142
Wheat flour (g)	28.142	28.142	28.142	28.142	28.142	26.142
Groundnut oil cake (g)	15.857	15.857	15.857	15.857	15.857	17.857
Fishmeal (g)	15.857	12.686	11.100	9.514	7.925	0
Brewer's spent yeast (g)	0	3.171	4.757	6.343	7.929	15.857
Fish oil[a] (ml)	2	2	2	2	2	2
Vegetable oil[b] (ml)	2	2	2	2	2	2
CMC[c] (g)	1	1	1	1	1	1
Vitamin[d] and Mineral[e] mixture (g)	2	2	2	2	2	2
Total amount (g)	99.998	99.998	99.998	99.998	99.998	99.998
Proximate composition						
Protein (%)	27.09	26.77	26.61	26.44	26.29	26.36
Lipid (%)	9.72	9.55	9.5	9.45	9.39	9.31
Energy (kJ)	1297.04	1292.86	1292.86	1288.67	1284.49	1288.67
Protein to Energy (P/E) ratio (% protein/kJ energy)	0.02	0.02	0.02	0.02	0.02	0.02
Moisture (%)	7.32	7.56	7.21	7.37	7.62	7.49

[a]Composition of fish oil (Approx. composition per capsule): Fish oil concentrate – 525 mg; cod liver oil – 268 mg; Fish oil – 257 mg; Blend providing Omega 3 nutrients – 400 mg (EPA – 199 mg and DHA – 161 mg)

[b]Composition of vegetable oil (soybean oil) (Approx. composition per 100 g): Protein and Carbohydrate−0 g; Fat−100 g; Total trans−fat content not more than − 0 g; Total saturated fat content not more than − 18 g; Total monounsaturated fat − 20–24 g; Total polyunsaturated fat−58–64 g; ALA−Omega 3 – 6−8 g; *Cholesterol − 0 mg; Added Vitamin A − 750 mcg; Added Vitamin D − 5 mcg; Added Vitamin E − 273 mcg

[c]CMC: Carboxy methyl cellulose

[d]To supply/100 g diet: Vitamin A (as acetate), 10000 IU; cholecalciferol, 1000 IU; thiamin mononitrate, 10.0 mg, riboflavin, 10 mg; pyridoxine hydrochloride, 60 mg; cyanocobalamin, 30 µg; 200 mg; ascorbic acids, 300 mg; α−tocopheryl acetate, 50 mg; biotin, 0.5 mg

[e]To supply/100 g diet: Calcium phosphate 258 mg; magnesium oxide, 120 mg; ferrous sulphate, 64.08 mg; manganese sulphate, 4.06 mg; copper sulphate, 6.78 mg; zinc sulpahte, 4.40 mg; sodium molybdate, 0.5 mg; sodium borate, 1.76 mg

experimental fishes from each tank were analyzed as per standard protocol [28] for calculating ANPU (%).

Blood and Serum Sample Collection

Individual fish were anaesthetized by using MS-222 before blood collection. Blood sample was collected from the caudal peduncle using a syringe (22 needle) rinsed in ethylene diamine tetra acetic acid (EDTA). The blood was collected in vials containing 0.5 mg EDTA anticoagulant. Whole blood was used for the estimation of haemoglobin, RBC and WBC count. For serum collection, blood was stored in vials without EDTA and centrifuged at 10,000 rpm for 20 min to separate the serum/plasma and stored at − 20 °C, which was later used for further analysis.

Haematological Parameters

The total count of red blood cells (RBC) and white blood cells (WBC) was estimated by diluting the blood with Haymen's fluid and Turk's diluting fluid, respectively [30–33]. Haemoglobin (Hb) was estimated using the cyanomethemoglobin method [34]. Packed cell volume (PCV) (Hematocrit) was estimated by centrifuging the hematocrit capillary tube [35].

Serum Biochemical Analysis

Total serum protein, albumin, lactate dehydrogenase (LDH), alanine amino-transferase (ALT) and aspartate amino-transferase (AST) and alkaline phosphatase (ALKP) content were analyzed by an automated blood biochemistry analyzer (EM-200, Erba-Transasia Biomedicals Ltd., Solan

H.P, India). Serum globulin was calculated as = total serum protein – serum albumin. Glucose and cortisol in serum was assayed using commercially available ELISA kit (Kit Rsbio, Shanghai, China and Kit Oxford Biochemical Research Inc., MI, USA).

Sample Collection for Digestive Enzyme Analysis

Fish were kept for a starvation period of 24 h before collection. The fish were sacrificed and dissected after employing anaesthetic agent tricaine methane sulfonate MS222 (70 mg/l, SIGMA-ALDRICH, France) and weighed. The whole digestive tract was removed and rinsed with cold Tris-HCl buffer (0.01 M, pH 7, SIGMA-ALDRICH, FRANCE). Samples were homogenized with glass (1 mmØ) beads in cold Tris-HCl buffer (0.01 M, pH7) using a homogenizer (Tissue Ruptor, Qiagen Hilden, Germany) at 5500 rpm and centrifuged (Remi Electrotechnik Ltd., India) at 15,000g for 15 min at 4 °C. Supernatants were collected and stored at −80 °C for further analysis. Amylase activity was assayed by using an amylase kit (NanjhingJianchen No. C016-1). The unit was defined as amylase in 1 mg protein which hydrolyzes 10 mg substrate (starch) in 30 min at 37 °C. That is considered 1 activity unit. Lipase activity was assayed by using a lipase kit (Nanjing Jianchen Bioengineering Institute, No.A054-1). The unit was defined as the lipase in 1 mg protein which hydrolyzes 1μmol substrate (triglycerides) at 37 °C. That is considered 1 activity unit. Total protease was measured using a non-specific assay [36], based on the use of casein as the substrate. For all the enzymatic activities, values obtained were normalized to the protein content of each sample, as estimated by [37] and expressed as specific activities (Units per mg of protein, U/mg protein).

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) using SPSS software (version 22). Multiple comparisons among means of individual diets of triplicate groups were made with Duncan's multiple range test [38] to evaluate the mean difference among the dietary groups at 0.05 significance levels. Means of triplicate experimental and initial groups ± SD are presented for the different diets. All parameters studied in the present experiment were subjected to principal component analysis using SPSS software.

Results

Growth

The water quality parameters of all the experimental tanks were maintained to keep no significant difference between the treatments. The growth attributes of fingerling fed with different levels of BSY are provided in Table 2. Weight gain (%) was increased significantly with an increase in the replacement of fishmeal by BSY up to D2 (554.5%)– D3 (502.0%). Fishmeal-based controlled diet gave higher final weight gain (441.5%) than D1 (406%), D4 (385.5%) and D5 (385%) replacement of fishmeal by BSY. FCR was found to be significantly lowest at D2 (1.63) replacement followed by D3 (1.75), D4 (1.86), D5 (1.89), D1 (1.90) replacement and control diet (1.92). The trend of changing ADG (%), PER, and SGR (%/day) in relation to the replacement of fishmeal by BSY indicated that 30–40% replacement is better than other replacement levels and fishmeal-based control diet for

Table 2 Growth attributes of *Labeo rohita* fed with different experimental diets

Parameters	Experimental diets					
	Control	D1	D2	D3	D4	D5
Initial weight (g)	2.0 ± 0.02	2.0 ± 0.02	2.0 ± 0.02	2.0 ± 0.02	2.0 ± 0.02	2.0 ± 0.02
Final weight (g)	10.83^{c} ± 0.06	10.12^{c} ± 0.06	13.09^{a} ± 0.05	12.04^{b} ± 0.06	9.71^{d} ± 0.08	9.70^{d} ± 0.08
Weight gain (%)	441.5^{c} ± 4.33	406.0^{d} ± 5.20	554.5^{a} ± 9.24	502.0^{b} ± 11.90	385.5^{e} ± 4.33	385.0^{e} ± 5.02
ADG (%)[a]	9.81^{c} ± 0.03	9.02^{d} ± 0.01	12.32^{a} ± 0.05	11.16^{b} ± 0.09	8.57^{e} ± 0.03	8.56^{e} ± 0.05
ADG (g/fish/day)[b]	0.109^{c} ± 0.001	0.100^{d} ± 0.001	0.136^{a} ± 0.002	0.124^{b} ± 0.002	0.095^{e} ± 0.001	0.095^{e} ± 0.001
SGR (%/day)[c]	0.94^{d} ± 0.03	1.80^{c} ± 0.05	2.09^{a} ± 0.03	1.99^{b} ± 0.01	1.76^{c} ± 0.01	1.75^{c} ± 0.01
FCR[d]	1.92^{c} ± 0.03	1.90^{c} ± 0.01	1.63^{a} ± 0.01	1.75^{b} ± 0.01	1.86^{c} ± 0.01	1.89^{c} ± 0.03
FCE[e]	0.52^{b} ± 0.01	0.53^{b} ± 0.01	0.61^{a} ± 0.01	0.57^{b} ± 0.01	0.54^{b} ± 0.01	0.53^{b} ± 0.01
PER[f]	1.86^{d} ± 0.01	1.88^{d} ± 0.01	2.19^{a} ± 0.03	2.04^{b} ± 0.01	1.92^{c} ± 0.01	1.89^{d} ± 0.01
ANPU (%)[g]	48.4^{c} ± 3.98	60.2^{b} ± 3.81	71.2^{a} ± 6.40	71.04^{a} ± 6.06	39.8^{d} ± 2.94	31.9^{e} ± 2.77
Survival (%)	93.6^{a} ± 2.60	94.3^{a} ± 2.08	96.7^{a} ± 1.90	95.2^{a} ± 2.25	92.9^{a} ± 2.08	92.4^{a} ± 2.94

Values are mean±SD. Values within the same row with different superscripts are significantly different (P<0.05)

[a]Average daily weight gain (%), [b]Average daily weight gain (g/fish/day), [c]Specific growth rate, [d]Feed conversion ratio, [e]Feed Conversion Efficiency, [f]Protein efficiency ratio, [g]Apparent net protein utilization

rohu. ADG (%), PER and SGR were at the highest levels of 12.32, 2.19 and 2.09 respectively at D2 followed by D3. The D4 and D5 replacement levels showed poor growth performance compared to the control diet. The ANPU (%) was 71.2 and 71.04 in D2 and D3, respectively and it was better compared to the control diet. The survival rate ranged from 92.4 to 96.7% in control and treatment groups.

Hematological Parameters

The present results confirm that the hematological parameters of rohu exhibit significant variations in response to changing levels of BSY in the diet. The haematological parameter of rohu fed with different levels of BSY replacing fishmeal is shown in Table 3. Total RBC was significantly higher ($p < 0.05$) in fish feed with D2 and D3 (1.9×10^6 mm^{-3}) diets followed by D4 (1.7×10^6 mm^{-3}) and D5 (1.6×10^6 mm^{-3}). The fishmeal-based control diet has the lowest amount of RBC (1.4×10^6 mm^{-3}). However, WBC count was found to be significantly higher ($p < 0.05$) in the group fed 100% replacement of fish meal by BSY (D5) (1.9×10^6 mm^{-3}) followed by D4, D1 and the control group. Fish fed with 30% replacement of fishmeal by BSY have significantly lower count of WBC (1.27). The total Hb content was significantly higher in fish fed with D2 and D3 diets (8.0–8.2 g dL^{-1}) as compared to fish with other diets. The Hb concentration significantly decreased in D4 and D5 diets. The value of PCV ranged from 28.1% in fish with D2 to 23.4% in fish with the D5 diet. There is a significant increase in PCV% of D2 in comparison to other diets including the control.

Biochemical Parameters

Serum biochemical parameters are portrayed in Fig. 1. The blood glucose level (µg dL^{-1}) was found to be significantly lower in fishes with D2 diet. It was highest and insignificantly different ($p < 0.05$) in groups fed with Control, D1 and D5. The mean serum alanine amino-transferase (ALT) level in fishes with D2 and D3 diets was 18 ± 0.28 to 19 ± 0.26 U L^{-1} which increased significantly to 28 ± 0.28 U L^{-1} in fishes with D4 diet. Control and 100% replacement of fishmeal by BSY (D5) diet showed similar ALT activities in fishes which is higher than the fish with D2 and D3 diet. Serum aspartate amino-transferase (AST) was found to be lowest like that of ALT in fishes fed with D2 diet (12 ± 1.84 U L^{-1}) which gradually increased significantly in fishes with

Table 3 Hematological parameters of *Labeo rohita* fed with different experimental diets

Blood parameters	Control	D1	D2	D3	D4	D5
Total RBC (10^6 mm^{-3})	$1.4^{e} \pm 0.001$	$1.5^{d} \pm 0.001$	$1.9^{a} \pm 0.002$	$1.9^{a} \pm 0.001$	$1.7^{b} \pm 0.001$	$1.6^{c} \pm 0.001$
Total WBC (10^6 mm^{-3})	$1.41^{d} \pm 0.001$	$1.49^{c} \pm 0.001$	$1.27^{f} \pm 0.001$	$1.31^{e} \pm 0.001$	$1.62^{b} \pm 0.001$	$1.90^{a} \pm 0.001$
Haemoglobin (g dL^{-1})	$7.0^{c} \pm 0.01$	$7.6^{b} \pm 0.03$	$8.2^{a} \pm 0.01$	$8.0^{a} \pm 0.01$	$7.0^{c} \pm 0.03$	$6.4^{d} \pm 0.01$
PCV (%)	$27.8^{a} \pm 0.40$	$25.2^{bc} \pm 0.53$	$28.1^{a} \pm 0.19$	$26.6^{b} \pm 0.36$	$24.2^{c} \pm 0.24$	$23.4^{d} \pm 0.36$

Values are Mean±SE. Values within the same row with different superscripts are significantly different (P<0.05)

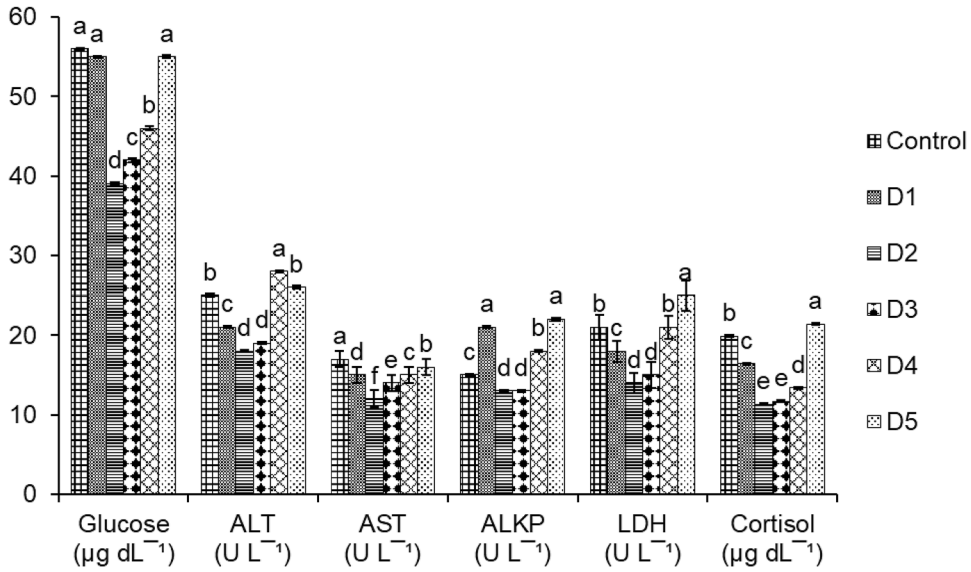

Fig. 1 The values of glucose, alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALKP), lactate dehydrogenase (LDH) and cortisol in the serum of *L. rohita* after 90 days experiment. Vertical bar denotes mean values and standard deviation; vertical bar with different superscripts within group are significantly different ($P < 0.05$)

D3, D4, D5 and control diets. The control diet showed the highest AST activity (17 ± 1.80 IU/l) in fish. Serum alkaline phosphatase (ALKP) activity was also found to be lowest in fishes with D2 and D3 diets. The control fishes have significantly higher ALKP activity than D2 and D3 diets. This activity increased gradually and significantly in fishes with D1, D4 and D5 diets. The serum lactate dehydrogenase (LDH) activity showed a similar trend to the ALKP enzyme. Its lowest activity was observed in fish with D2 and D3 and gradually increased ($p < 0.05$) in fishes with D4 and D5 diets. The control diet showed similar LDH activity to that of fishes with D4 diet. Serum cortisol level was observed to be lowest in fishes with diet D2 and D3 and increased significantly ($p < 0.05$) in fish with D4 and D5 diets. The fish with control and D1 diet has significantly lower ($p < 0.05$) serum cortisol levels than the fish with D5 diet. Fish with D1 diet also have higher cortisol levels than the fishes with D2 and D3. The total serum protein, albumin and globulin (Fig. 2) were found to be significantly higher in the D2 group followed by D3, D4 and Control groups.

Digestive Enzymes

The intestinal enzyme activities were shown in Table 4. The highest amylase activity was shown in fish with D1 (1.38 U mg protein^{-1}) which decreased significantly ($p < 0.05$) in the fish fed with D2, D3 and D4 diets. The control diet-fed fish had similar ($p > 0.05$) amylase activity (1.18 U mg protein^{-1}) with that of D3. There was not much variation ($p > 0.05$) in protease activity of fish fed with BSY based experimental diets (D1–D5). The control group had significantly lower ($p < 0.05$) protease activity than the experimental fish. lipase activity was observed to be highest with D2 diet (1.52 U mg protein^{-1}) which further decreased with the increase in replacement level of BSY from D3 to D5 diet. The control diet showed a moderate level of lipase activity which was found to be higher than the fish with D5 diet.

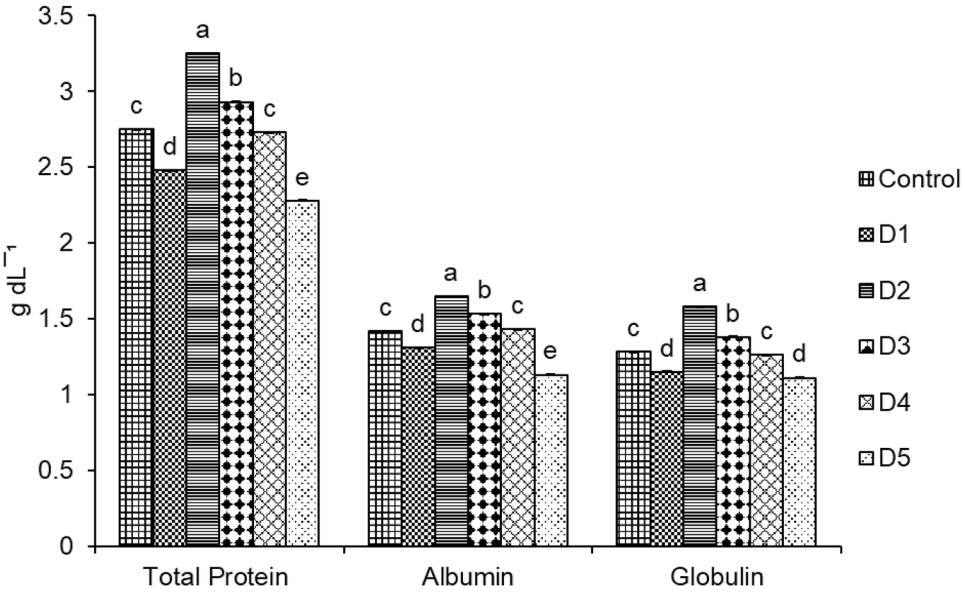

Fig. 2 The values of total protein, albumin and globulin in the serum of *L. rohita* after 90 days experiment. Vertical bar denotes mean values and standard deviation; vertical bar with different superscripts within group are significantly different ($P < 0.05$)

Table 4 Digestive enzyme analysis of *Labeo rohita* fed with different experimental diets

Parameters	Experimental diets					
	Control	D1	D2	D3	D4	D5
Amylase (U mgprotein^{-1})	$1.18^{c} \pm 0.05$	$1.38^{a} \pm 0.01$	$1.29^{b} \pm 0.03$	$1.18^{c} \pm 0.01$	$1.13^{d} \pm 0.01$	$1.19^{c} \pm 0.03$
Protease (U mgprotein^{-1})	$2.02^{b} \pm 0.05$	$3.04^{a} \pm 0.06$	$3.05^{a} \pm 0.05$	$3.01^{a} \pm 0.03$	$3.00^{a} \pm 0.03$	$3.02^{a} \pm 0.03$
Lipase (U mgprotein^{-1})	$1.12^{d} \pm 0.05$	$1.38^{b} \pm 0.01$	$1.52^{a} \pm 0.03$	$1.26^{c} \pm 0.01$	$1.27^{c} \pm 0.01$	$1.06^{e} \pm 0.01$

Values are Mean±SE. Values within the same row with the same superscripts are not significantly different (P<0.05)

Table 5 Principal component analysis of growth attributes, digestive enzymes, and biochemical parameters of *Labeo rohita* fed with different experimental diets

Rotated component matrix[a]			
	Component		
	1	2	3
FW	0.885	0.324	0.241
WG	0.885	0.324	0.241
ADWG Percent	0.885	0.325	0.240
ADWG	0.888	0.318	0.236
FCR	−0.671	−0.699	−0.140
FCE	0.651	0.708	0.173
PER	0.676	0.682	0.153
SGR	−0.030	0.951	0.286
ANPU	0.707	0.283	0.594
Survival	0.765	0.380	0.515
RBC	0.445	0.874	−0.072
WBC	−0.882	−0.028	−0.380
Haemoglobin	0.692	0.409	0.553
PCV	0.941	−0.219	0.240
Glucose	−0.616	−0.774	0.072
Serum Total Protein	0.916	0.341	0.039
Serum Albumin	0.899	0.309	0.084
Serum Globulin	0.894	0.402	0.044
ALT	−0.582	−0.334	−0.671
AST	−0.473	−0.802	−0.335
ALKP	−0.975	−0.118	0.109
LDH	−0.724	−0.403	−0.505
Cortisol	−0.547	−0.693	−0.157
Amylase	−0.064	0.026	0.976
Protease	−0.342	0.902	0.252
Lipase	0.417	0.524	0.606

Extraction Method Principal Component Analysis, *Rotation Method* Varimax with Kaiser Normalization

[a]Rotation converged in 5 iterations

Principal Component Analysis (PCA)

In the present study, the obtained PCA was rotated using varimax rotation with Kaiser normalization. Table 5; Fig. 3 depict the rotated PC matrix composed of a factor loaded by each biological parameter evaluated in the present study. Three PC were obtained with Eigenvalue > 1. PC1, PC2 and PC3 accounted for an Eigenvalue of 18.80, 3.58 and 2.18 respectively. In PC1, total variation accounted for 72.34%, where a strong positive correlation was observed among FW, WG, ADWG%, ADWG, survival, PCV, total serum protein, serum albumin and serum globulin (Table 5). PC2 obtained 13.77% of the total variation with a strong positive correlation between SGR, RBC and protease. Likewise, PC3 obtained 8.39% of the total variation. A negative correlation was found for parameters like WBC, ALT, AST, LDH and cortisol.

Discussion

Fishmeal replacement has been a prime objective in aquaculture-based nutrition studies among global scientists since this protein source become a commodity of scarce and expensive nature [9]. Several earlier research has been conducted to replace fishmeal with a variety of plant-based protein-rich ingredients to reduce feed costs and to find out the efficacy of different alternative feed ingredients. Some of the other protein sources that have been experimented with as alternatives for fishmeal are meat trimmings [39], insect-based meal [40], and algal meal [41]. Mishra et al. [42] studied the growth performance and survival of rohu using algae-based diets without using fishmeal. Similarly, The effect of *Azolla* supplementation have been studied on the growth of rohu without any fishmeal [43]. The growth parameters of rohu using biofloc as a component in feed has also been observed [44]. The use of brewery waste as livestock feed has been a common practice since the production of beer has risen exponentially [45]. With ample production of brewery waste in developing countries like India, this alternative protein source for fishmeal replacement can be widely accepted in aquafeed industries [19]. BSY is a nutrient-rich waste product of the brewer's industry and is available in plenty at a low cost. However, its use as a fish feed ingredient is not attempted much. In the present study, *Labeo rohita* were reared under controlled environmental conditions and supplemented with different levels (20%, 30%, 40%, 50% and 100%) of BSY-based experimental diets to study its efficacy. The growth of fish is a complex process influenced by several parameters like fish species cultured, the nutritional composition of feed supplemented, and the rearing environment. In the present study water quality was maintained at a constant and benevolent level in all treatments. So, the variation in the growth of rohu in this study is presumed to be due to the feed ingredient quality and compositional status as iso-nitrogenous (27% protein) and isocaloric (190 kcal/100 g) diet was fed to all dietary treatment groups. The BSY inclusion level at 50% and beyond has lowered the growth performances of *L. rohita* was affected in comparison to the control. This might be attributed due to the presence of high nucleic acid content in BSY which may have depressed the feed intake of *L. rohita*. The feed intake ultimately might have affected growth parameters of fish which resulted in poor feed utilization. In earlier studies, higher than 50% inclusion of BSY in the diet has been found to affect the growth of tilapia [46]. On the contrary, complete replacement of fishmeal with brewery soluble in diet

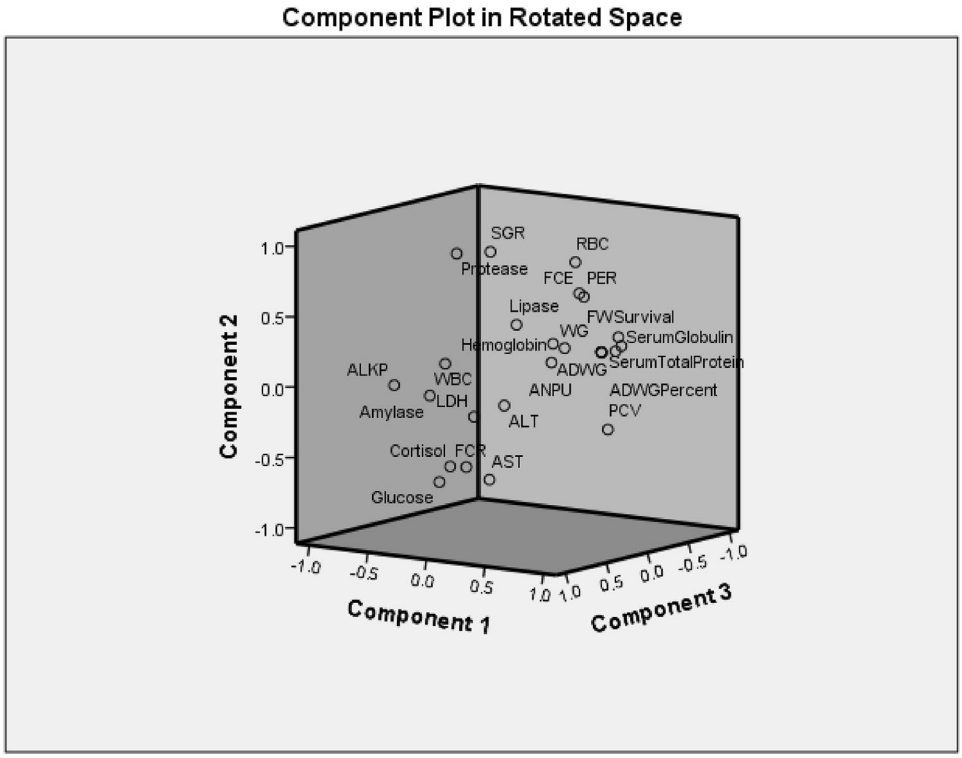

Fig. 3 Principal component analysis of different parameters of *Labeo rohita* fed with different experimental diets

did not affect the growth of channel catfish [47]. This might be attributed due to the different experimental fish species.

The growth rate of rohu was earlier studied in an isonitrogenous (32% protein) and iso-caloric (352 kcal/100 g) diet containing individually four algae, which showed that dietary inclusion of algae at 40% obtained better growth rate than higher inclusion level [42]. The present study on rohu with 30% replacement of fishmeal with BSY gave the highest growth (554.5%) followed by 40% replacement (502%) at a protein level of 26.61%. Using 20% fermented *Pistia* leaves (Pl) at 35% protein level, *L. rohita* got the highest growth of 106.3% [48]. This shows the present study using BSY at a lower protein level gives better growth in rohu as compared to algae-based diets or control diet. Feeding rohu level at 50% wet floc can maximize the values of net fish yield up to 1810 kg/ha [44]. The present study suggests proper energy level in the diet supports growth which is in agreement with an earlier report [42]. The highest growth in giant freshwater prawns (*Macrobrachium rosenbergii*) was obtained by replacing fishmeal with brewers spent yeast up to a level of 60% in clear water systems and 40% replacement in biofloc systems [49]. In the present study on rohu 30% replacement of fishmeal by BSY gave the highest growth which is in agreement with the earlier author's report on the biofloc system. Another study have shown that intact and extracted yeast (*S. cerevisiae*) can replace up to 40% of crude protein of fishmeal which suggests that intact *S. cerevisiae* yeast is a promising protein source for Arctic char [50]. A substitution level of 30% yeast has been advocated for koi carp (*Cyprinus carpio*) [51] while 25% seems ideal for rainbow trout (*Oncorhynchus mykiss*) [52]. Tilapia (*Oreochromis niloticus*) has been reported to utilize diets with 20% fishmeal substituted with BSY effectively with a higher percentage eliciting deleterious effects on growth [53]. The digestibility of spent yeast by catfish has been reported as 35% [54]. The present study on rohu is in agreement with earlier reports that 30% substitution of fishmeal with BSY showed the highest growth performance even better than fishmeal-based controlled diet and higher substitution level decreased growth. The FCR observed in the present study on rohu was 1.63 and 1.75 with 30% and 40% replacement of fish meal by BSY respectively, which is better than the fishmeal-based control diet (1.92). The FCR observed for rohu with a diet containing 50% and 100% BSY was found to be a little higher but comparable to that reported for rohu with algae-based diets (1.2 with *Westleopsis* diet and 1.3 with *Nostoc* and *Spirulina* diet) [42]. With biofloc, the FCR was reported to be more than 2 [44]. The FCE, PER, SGR (%/day), and ANPU (%,) were observed to be better in 30% replacement of fishmeal with BSY followed by 40% replacement which is better than fishmeal-based control diet in the present study on rohu. This is in agreement with the previous report [26]. Analysis of blood indices has proven to be a valuable approach for determining the health status of fish which provides reliable information on metabolic conditions. The haematological study includes blood and its different components which are

important in diagnosing the structural and functional status and are also important for the evaluation of the physiological condition of fish. Evaluation of hematology involves the determination of total RBC and WBC count, Hb concentration, hematocrit and PCV. [55]. In an earlier study, it was observed changes in RBC-dependent parameters i.e. RBC count, Hb and PCV with changes based on feed supplementation as an adaptation on the part of rohu to cope with change in feed ingredient [56]. The inclusion of BSY has shown changes in hematological parameters in earlier studies on gilthead seabream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*) [57–59]. The tannin present in *Jatropha* affected the absorption of iron and cyanocobalamine and caused low Hb formation and fragmentation of erythrocytes in rohu [60]. The most common blood variables consistently influenced by the presence of probiotics in the rohu diet are PCV and Hb levels [61]. In the present study, BSY or *Saccharomyces cerevisiae* in the diet was found to improve total RBC count, Hb and PCV and reduce WBC count by up to 30% through replacement of fishmeal. Inclusion of BSY in the diet showed significant variations which may be due to the effect of yeast on rohu immunology. Two rohu groups fed with the diet supplemented with dead *Saccharomyces cerevisiae* and both live *Bacillus subtilis* and *Saccharomyces cerevisiae* showed a significant increase in PCV compared to fish fed the control diet [61]. In the present study, the total PCV value in rohu was observed to be higher in the fishmeal-based control diet D2 and D3 diet which may be due to less stressful conditions. A positive correlation between total leucocyte count and the feeding status of the rohu has been observed in earlier study [56]. In our present study total WBC count in rohu was least with D3 diet which gradually increased to D5 diet which showed the direct effect of fishmeal replacement by BSY on WBC count, which is similar to earlier findings [56]. The low WBC count with a D3 diet may be related to the immunity of the rohu.

In rohu, males' total protein and albumin content were higher compared to females [56]. The authors reported that the serum protein in fish is dependent on the environmental temperature and feeding regime and it can be influenced by other factors like age, sex, disease, and dietary protein. The total protein concentration of serum and the circulating mobile proteins including albumin and globulin play a major role in the immune response of fish showing the nutritional status and fish health [62]. Replacing fishmeal meal with soya protein isolate in the diet of *Acipensers chrenckit* showed low serum protein level [63]. Serum total protein levels of rohu subjected to transportation stress showed a decreasing trend, indicating the breakdown of protein [64]. In the present study, replacing fish meal with BSY up to 30% increased serum total protein, albumin and globulin level and thereafter these parameters gradually decreased up to 100% replacement of fishmeal with BSY. This may be due to the yeast which acted as a stimulant up to a particular level.

The high blood glucose level in fish has been considered a secondary level of stress indicator [65–68] and also indicates high energy demanding condition [19]. It is reported that during stress catecholamine and cortisol secretion from the head kidney increases which in turn elevates serum glucose levels for energy production to mitigate the stressful condition [69]. In Nile tilapia it has been reported that higher blood glucose levels obtained when fed with *Jatropha platyphylla* kernel meal-based diet [70]. In rohu higher blood glucose levels reported when fed with 20% *Jatropha* concentrate compared to other dietary groups which may be due to phorbol ester-induced stress [60]. The blood glucose levels could modulate cortisol release in fish and high glucose levels could result in high cortisol levels [71]. In the present study blood glucose and cortisol levels were found to be significantly lowest with D3 diet compared to fishmeal based control diet which increase gradually with the increase in replacement of fishmeal with BSY (D4–D6) diets. This indicated that stress level due to higher concentration of nucleic acid in higher BSY inclusion level was observed in rohu, since the formation of uric acid by metabolization of nucleic acid is harmful for fish. This observation has also been found in previous studies on inclusion of BSY in fish diet [72]. Blood glucose is lowest with D3 diet in rohu which indicates that BSY is a suitable ingredient to replace fishmeal upto 30% level.

Alanine transaminanse (ALT) and Aspartate trasaminanse (AST) are the two most important amino acid metabolizing enzymes which can shed light on the intensity of fish amino acid metabolism. The tissue distribution of AST and ALT in *Channa punctatus* was studied by [73]. The authors reported the specific activities of AST and ALT/mg protein in the liver, muscle, brain, kidney and gill. In the present study, the AST and ALT activities were studied in the blood and intestine. In *Channa striatus* it was reported that AST and ALT activities are affected by environmental temperature [74]. Change in ALT and AST activities in different tissues of *Channa punctatus* in an annual cycle was recorded and studied earlier [27]. In the present study, the ALT and AST activities were studied in blood and intestine. In earlier study, it has also been observed the effect of stocking density, transportation stress and dietary inclusions on AST and ALT activities [64]. As liver cells damage or die, ALT leaks out into the blood. Serum ALT is therefore a good marker of acute hepatic damage [75]. Similar activities of AST like ALT in rohu was reported that both enzymes are associated with liver parenchymal cells [64]. The effect of stocking density on AST was studied [76] where 30 fingerling, each of 2 g body weight, were stocked in 250 L water for all the experimental and control groups to avoid stocking density induced stress effect. In another earlier study, observed a

significant reduction of liver AST and ALT activities in rohu due to dietary fucoidan [77]. However, an increase in muscle ALT of rohu was noticed with the fucoidan diet. In the present study on rohu, ALT activity in serum is more compared to AST activity. AST and ALT redistribute amino nitrogen among the amino acids forming new amino acids with the amino group from the pre-existing ones [77]. In present study, both the enzyme activity in blood were significantly lower with D3 and D4 diet which may be that the stress level is less and metabolism in the liver leads to sparing of protein to be used as an energy source [78]. Therefore, the result suggests that the test diets (D2 and D2) protected and prevented damage to the liver plasma membrane so that the leakage of the enzyme from the liver to serum is less. ALT and AST were expressed better in *Pangasianodon hypophthalamus* fed continuously with 35% protein compared to alternate feeding of high (35%) and low (25%) protein diet [79]. In our experiment the protein level of 27% was fed to rohu with different replacement level of fishmeal by BSY and enzyme activities were observed.

Serum alkaline phosphatase (ALKP) of rohu in the present study was also lowest with D2 and D3 diets compared to the control diet. Isosoamal enzyme ALKP present in liver and bone cells participates in the skeleton mobilization of aquatic animals and performs membrane transport activity [76]. The increase in ALKP concentration in blood with the control diet and higher replacement level of fishmeal by BSY in this study may be due to increased osteoblastic activity. However, with D2 and D2 diets, this activity is less. A higher value of ALKP at a stocking density of 10 fish/m^3 was obtained which showed that stocking density had affected ALKP [76]. Alkaline phosphatase activity was reported to be an indicator of the intensity of nutrient absorption in enterocytes of fish [80]. Debnath et al. [81] did not find any significant change in ALKP activity in rohu fed with different crude protein levels. A positive correlation was reported between ALKP activity and the growth rate of Atlantic cod [82]. In carnivorous fish higher ALKP activity was reported compared to herbivorous fish [83]. ALKP in rohu was reported to be less significant in the growth and nutrient absorption [81]. A significant increase in serum ALKP activity in channel catfish was reported with increased availability of phosphorus in the diet [84]. The higher protein levels in the diet increases phosphorus due to high fishmeal levels in the diet [79]. In the present study, ALKP activity was observed to be less at 30–40% replacement of fishmeal with BSY which is related to the growth of rohu as nutrient absorption was better at that level.

It has been demonstrated that plasma lactate levels increased in fish subjected to stress as energy was exhausted in a short time and fish need to generate energy through anaerobic metabolism [85]. Injecting grass carp with glucose at 0.5 and 1.0 mg/g BM showed lower plasma lactate levels but higher LDH activity suggesting that high blood glucose levels might increase lactate generation [86]. They reported that handling grass carp resulted in increased plasma levels of glucose, cortisol, lactate and plasma LDH activity. In the present study on rohu plasma glucose, cortisol and LDH activity was decreased with D2 and D3 diet compared to the control diet. This shows that diet affects these blood parameters and these diets decrease stress levels in fish. In a fishmeal-based control diet, these blood parameters are significantly higher which suggests that BSY is better than fishmeal as a dietary ingredient. The LDH activity increased in higher packing density, due to elevation in anaerobic catabolism of blood cortisol and damage of liver and muscle tissue because of stress [64].

The knowledge of the feeding habits of different fish species associated with enzyme activities in the digestive tract is important to provide an appropriate diet. It is necessary to know the digestive capability of reared species for adapting dietary formulation to the functionality of the digestive tract [87]. As digestive enzymes can be used as surrogate parameters to predict the ability of fish to use different nutrients, in vitro measurements of digestive enzyme activities may allow adapting artificial feeding to the nutritional needs of fish [88]. Fish may adapt their metabolic functions to the dietary substrates, through regulation in enzyme secretion, to improve the utilization of feed ingredients. The digestive enzymes play a significant role in the hydrolysis of protein, lipid and carbohydrates. Digestion of ingested nutrients starts with the effect of digestive enzymes in the stomach and continues in the intestine by the digestive enzymes secreted by the pancreas such as trypsin, chymotrypsin, amylase, and lipase [89]. In the present study, there is no difference in the protease enzyme activity among the experimental groups. But fishmeal based control diet had significantly lower protease activity. The determination of protease activity of fish gut and liver elucidate the level of protein utilization by fish [90]. Secretion of proteolytic enzymes in fish depends upon the nutrient composition of the diet, level of stomach filling and protein intake by fish [91]. In the intestine of *Pangasianodon* fed alternatively with 35% and 25% crude protein showed better protease activity which is at par with the activity of continuous feeding of 35% protein diet [79]. In the present study rohu fingerlings were fed continuously with 27% crude protein diet both the experimental and control group, which is in agreement with report of [81] on rohu feeding 25% crude protein diet. This study shows that diets in which fishmeal is replaced with BSY protein utilization is better.

The amylase activity of the stomach of giant cat fish (*Pangasianodon gigas*) was highest at optimal temperature between 25 and 30 °C [92]. In the present study the temperature is maintained at 27 °C. Tok et al. [79] positively correlated the activity of amylase with carbohydrate level in

diet. Some earlier studies did not find any influence of level of dietary carbohydrate on the amylase activity [81, 93] . In the present study higher amylase activity was observed with the diet in which fishmeal is replaced by 20% BSY. But carbohydrate level in the diets was not changed. The present study is in agreement with earlier reports. The activity of lipase is based on the level and source of lipid in the diet [94]. Tok et al. [79] reported less variation in the intestinal lipase activity of *Pangasianodon hypophthalamus* with continuous and alternate feeding of different protein diets with the same level of lipid in the diet. Debnath et al. [81] also found the same lipase activity in rohu due to a constant lipid content diet. In the present study, the lipid is kept at a constant level (9.5%). It is also observed that at a lower inclusion level of BSY (20% and 30%) higher the lipase activity which may be due to the different levels of inclusion of BSY. The effect of diet composition on digestive enzyme activities was reported in earlier study [95]. Among the different ingredients used for diet formulation by the author, the highest amount of lipid was found in the almond oil cake with the highest lipase activity in rohu.

In earlier studies [96, 97], PCA interpretation-based correlation among various biochemical parameters and growth attributes has been conducted. PCA analysis in the present study confirmed that some biochemical parameters were strongly correlated which indicates that these parameters are interdependent.

Conclusion

From our study it was found that the rohu fingerling fed diet D2 (30% fish meal replacement by Brewer's spent yeast) had significantly higher growth, feed utilization efficiency and nutrient gain as compared to other diet fed groups including control. The hematological parameters were significantly higher, but the total WBC and glucose levels were lower in fish fed diet D2 groups than the fish fed other diets. The blood ALT, AST, ALKP, LDH and Cortisol levels of fish were significantly lower in diet D2 than in the other diets. The intestinal enzyme activity showed that the rohu fed diet D2 had significantly higher protease and lipase activity and lower ALT and AST activity. Therefore, from our study it is concluded that the fish meal can be replaced 30% by Brewer's spent yeast based on growth, nutrient and feed utilization and overall health and immunity status of fish. The use of Brewer's spent yeast as non-conventional feed ingredient will help in formulating the nutritionally balanced cost-effective diet for carp.

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Author Contributions KS and HSS contributed to the study conception and design. Material preparation, data collection and analysis were performed by DP, AU, KNM, and SSG. The first draft of the manuscript was written by DP, HSS, AU and BS, SN, SKP and KS commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on request.

Declarations

Conflict of Interest The authors have no relevant financial or non-financial interests to disclose.

Research Involving Human and/or Animal Participants The authors followed all the applicable international, national and/or institutional guidelines for the care and use of animals.

Consent to Participate The authors give consent for participation.

Consent for Publication The authors give consent for publication.

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